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PATENT- OG VAREMÆRKESTYRELSEN

AN ALLERGEN DOSAGE FORM

Technical Field

This invention relates to allergen vaccines and in particular to fast dispersing solid allergen vaccine dosage forms and a method for preparing such dosage forms.

Background of the Invention

Allergy is a major health problem in countries where Western lifestyle is adapted. Furthermore, the prevalence of allergic disease is increasing in these countries. Although allergy in general may not be considered a life-threatening disease, asthma annually causes a significant number of deaths. An exceptional prevalence of about 30% in teenagers conveys a substantial loss in quality of life, working days and money, and warrants a classification among major health problems in the Western world.

Allergy is a complex disease. Many factors contribute to the sensitisation event. Among these is the susceptibility of the individual defined by an as yet insufficiently understood interplay between several genes. Another important factor is allergen exposure above certain thresholds. Several environmental factors may be important in the sensitisation process including pollution, childhood infections, parasite infections, intestinal microorganisms, etc. Once an individual is sensitised and the allergic immune response established, the presence of only minute amounts of allergen is efficiently translated into symptoms.

The natural course of allergic disease is usually accompanied by aggravation at two levels. Firstly, an progression of symptoms and disease severity, as well as disease progression, for example from hay fever to asthma. Secondly, dissemination in offending allergens most often occurs resulting in allergic multi-reactivity. Chronic inflammation leads to a general weakening of the mucosal defense mechanisms resulting in unspecific irritation and eventually destruction of the mucosal tissue. Infants may become sensitised pri-

marily to foods, i.e. milk, resulting in eczema or gastrointestinal disorders; however, most often they outgrow these symptoms spontaneously. These infants are at risk of developing inhalation allergy later in their lives.

The most important allergen sources are found among the most prevalent particles of a certain size in the air we breathe. These sources are remarkably universal and include grass pollens and house dust mite faecal particles, which together are responsible for approximately 50% of all allergies. Of global importance are also animal dander, i.e. cat and dog dander, other pollens, such as mugwort pollens, and micro-fungi, such as Alternaria. On a regional basis yet other pollens may dominate, such as birch pollen in Northern and Central Europe, ragweed in the Eastern and Central United States, and Japanese cedar pollen in Japan. Insects, i.e. bee and wasp venoms, and foods each account for approximately 2% of all allergies.

Allergy, i.e. type I hyper-sensitivity, is caused by an inappropriate immunological reaction to foreign non-pathogenic substances. Important clinical manifestations of allergy include asthma, hay fever, eczema, and gastro intestinal disorders. The allergic reaction is prompt and peaks within 20 minutes upon contact with the offending allergen. Furthermore, the allergic reaction is specific in the sense that a particular individual is sensitised to particular allergen(s), whereas the individual does not necessarily show an allergic reaction to other substances known to cause allergic disease. The allergic phenotype is characterized by a pronounced inflammation of the mucosa of the target organ and by the presence of allergen specific antibody of the IgE class in the circulation and on the surfaced of mast-cells and basophils.

An allergic attack is initiated by the reaction of the foreign allergen with allergen specific IgE antibodies, when the antibodies are bound to high affinity IgE specific receptors on the surface of mast-cells and basophils. The mast-cells and basophils contain preformed mediators, i.e. histamine, tryptase, and other substances, which are released upon cross-linking of two or more receptor-bound IgE antibodies. IgE antibodies are cross-linked by the simulta-

neous binding of one allergen molecule. It therefore follows that a foreign substance having only one antibody binding epitope does not initiate an allergic reaction. The cross-linking of receptor bound IgE on the surface of mast-cells also leads to release of signaling molecules responsible for the attraction of eosinophils, allergen specific T-cells, and other types of cells to the site of the allergic response. These cells in interplay with allergen, IgE and effector cells, lead to a renewed flash of symptoms occurring 12-24 hours after allergen encounter (late phase reaction).

Allergy disease management comprises diagnosis and treatment including prophylactic treatments. Diagnosis of allergy is concerned with by the demonstration of allergen specific IgE and identification of the allergen source. In many cases a careful anamnesis may be sufficient for the diagnosis of allergy and for the identification of the offending allergen source material. Most often, however, the diagnosis is supported by objective measures, such as skin prick test, blood test, or provocation test.

The therapeutic options fall in three major categories. The first opportunity is allergen avoidance or reduction of the exposure. Whereas allergen avoidance is obvious e.g. in the case of food allergens, it may be difficult or expensive, as for house dust mite allergens, or it may be impossible, as for pollen allergens. The second and most widely used therapeutic option is the prescription of classical symptomatic drugs like anti-histamines and steroids. Symptomatic drugs are safe and efficient; however, they do not alter the natural cause of the disease, neither do they control the disease dissemination. The third therapeutic alternative is specific allergy vaccination that in most cases reduces or alleviates the allergic symptoms caused by the allergen in question.

Conventional specific allergy vaccination is a causal treatment for allergic disease. It interferes with basic immunological mechanisms resulting in persistent improvement of the patients' immune status. Thus, the protective effect of specific allergy vaccination extends beyond the treatment period in

contrast to symptomatic drug treatment. Some patients receiving the treatment are cured, and in addition, most patients experience a relief in disease severity and symptoms experienced, or at least an arrest in disease aggravation. Thus, specific allergy vaccination has preventive effects reducing the risk of hay fever developing into asthma, and reducing the risk of developing new sensitivities.

The immunological mechanism underlying successful allergy vaccination is not known in detail. A specific immune response, such as the production of antibodies against a particular pathogen, is known as an adaptive immune response. This response can be distinguished from the innate immune response, which is an unspecific reaction towards pathogens. An allergy vaccine is bound to address the adaptive immune response, which includes cells and molecules with antigen specificity, such as T-cells and the antibody producing B-cells. B-cells cannot mature into antibody producing cells without help from T-cells of the corresponding specificity. T-cells that participate in the stimulation of allergic immune responses are primarily of the Th2 type. Establishment of a new balance between Th1 and Th2 cells has been proposed to be beneficial and central to the immunological mechanism of specific allergy vaccination. Whether this is brought about by a reduction in Th2 cells, a shift from Th2 to Th1 cells, or an up-regulation of Th1 cells is controversial. Recently, regulatory T-cells have been proposed to be important for the mechanism of allergy vaccination. According to this model regulatory T-cells, i.e. Th3 or Tr1 cells, down-regulate both Th1 and Th2 cells of the corresponding antigen specificity. In spite of these ambiguities it is generally believed that an active vaccine must have the capacity to stimulate allergen specific T-cells, preferably TH1 cells.

Specific allergy vaccination is, in spite of its virtues, not in widespread use, primarily for two reasons. One reason is the inconveniences associated with the traditional vaccination programme that comprises repeated vaccinations i.a. injections over a several months. The other reason is, more importantly, the risk of allergic side reactions. Ordinary vaccinations against infectious

agents are efficiently performed using a single or a few high dose immunizations. This strategy, however, cannot be used for allergy vaccination since a pathological immune response is already ongoing.

Conventional specific allergy vaccination is therefore carried out using multiple subcutaneous immunizations applied over an extended time period. The course is divided in two phases, the up dosing and the maintenance phase. In the up dosing phase increasing doses are applied, typically over a 16-week period, starting with minute doses. When the recommended maintenance dose is reached, this dose is applied for the maintenance phase, typically with injections every six weeks. Following each injection the patient must remain under medical attendance for 30 minutes due to the risk of anaphylactic side reactions, which in principle although extremely rare could be life-threatening. In addition, the clinic should be equipped to support emergency treatment. There is no doubt that a vaccine based on a different route of administration would eliminate or reduce the risk for allergic side reactions inherent in the current subcutaneous based vaccine as well as would facilitate a more widespread use, possibly even enabling self vaccination at home.

Attempts to improve vaccines for specific allergy vaccination have been performed for over 30 years and include multifarious approaches. Several approaches have addressed the allergen itself through modification of the IgE reactivity. Others have addressed this route of administration.

The immune system is accessible through the oral cavity and sublingual administration of allergens is a known route of administration.

Conventionally allergy vaccine using the oromucosal route consists of the up to daily dosing of a solution of the allergen. In comparison, the therapeutic (accumulated) maintenance doses given exceeded the maintenance of the comparable subcutaneous dose by a factor 5-500. Obvious drawbacks of this dosage form and route of administration are the problems associated with

accurate and uniform self administration of the correct dose by the patient (several drops may have to be given, uniformity of the individual drops, application site accuracy, etc.). There is additionally a need to refrigerate the drug and include preservatives in the formulation.

Netien et al.: "Galenica 16 – Médicaments homéopathiques" ed. 2, 1986, pages 77-99 discloses a liquid solution impregnated onto a solid particulate (granules) or conventional compressed tablets of lactose, saccharose or a mixtures of these for sublinqual administration of medicaments such as allergens. However these dosage forms are associated with serious drawbacks, such as the impregnation procedure.

DD-A.0 107 208 discloses a process for preparing a conventional compressed tablet containing an allergen. Upon administration the tablet is dissolved by the saliva and the allergen is then absorbed through the mucosa of the oral cavity. The formulation contains a water insoluble excipient, namely talcum as well as paraffin and fatty acids which is not desirable since it will leave an unpleasant remnant in the mouth of the patient. Moreover, the friction during the tabletting process may be detrimental to the physical stability of the allergens.

EP 278 877 discloses a pharmaceutical composition for sublingual use, where a solid support is coated with a solution of an allergen. The resulting formulation is alleged to disintegrate rapidly, but not instantaneously. However, there is no disclosure of how to achieve the objective. Moreover, the formulation contains reducing sugars in the form of lactose, which are prone to react with allergens.

In order to ensure that as much as possible of an administered dose of a certain allergen is presented to the mucosa of the oral cavity and additionally that the contact time of the disintegrated product with the mucosa is maximised, it is very important that the dosage form disintegrates instantaneously upon contact with the saliva of the oral cavity. Fast dispersing solid dosage

forms, which readily release the active ingredient in the oral cavity are known in the art.

U.S. patent No 4,371,516 discloses pharmaceutical dosage forms containing active ingredients, which disintegrate rapidly in water. The pharmaceutical dosage forms comprise an open matrix network of carrier material, which disintegrate within 10 seconds.

A freeze-dried fish gelatine based carrier as disclosed in WO 00/61117 is designed to release the active ingredient instantaneously upon contact with saliva when administered in the oral cavity.

A freeze-dried modified starch carrier as disclosed in WO 00/44351 is designed to release the active ingredient instantaneously upon contact with saliva when administered in the oral cavity.

WO 99/21579 discloses a fast-dispersing dosage form comprising a vaccine and an adjuvant for oral use.

WO 02/13858 discloses fast dissolving pharmaceutical composition containing vaccines in the form of a fast dissolving "cake" for oral use. The object of WO 02/13858 appears to be to provide viral or bacterial vaccines that will stay intact in the gastrointestinal tract. This is achieved by protecting the antigen against the acidic content of the stomach by incorporating antacids such as calcium carbonate into the cake.

WO 00/51568 discloses a fast-disintegrating compressed low friability tablet that is designed to dissolve in the mouth in contact with saliva in less than 30 seconds forming an easy-to-swallow suspension.

It is alleged in U.S. patent No 4,371,516 that the formulation is useful for oral vaccines, in the case of WO 00/61117, WO 00/44351, WO 99/21579 and WO 02/13858 it is also alleged that the inventions are directed to non-infections

immuno-modulated conditions such as systemic allergic conditions e.g. hay-fever. However, there is no disclosure in the form of technical information or examples of how a fast dispersing allergen vaccine solid dosage form can be manufactured. As an example, there are no indications of an appropriate dosage of a certain allergen in any of the disclosed formulation. It is very important to administer a correct dosage of an allergen to a patient, because a too high dose may induce an anaphylactic shock in the patient. Further no recognition of and indications of appropriate measures in relation to stability or friability of such formulation are given.

Summary of the invention

The present invention concerns a fast dispersing non-compressed solid allergen dosage form for oromucosal administration characterized in that the dosage form is stable, sufficiently robust and does not release hazardous amounts of residue upon handling by the patient and comprises at least one allergen and a matrix.

The current invention relates to a stable, fast dispersing, low friable, non-compressed solid allergen vaccine dosage form suitable for oromucosal administration of allergen comprising

(a) a matrix

(b) at least one allergen

In one preferred embodiment of the invention, the solid dosage form comprises fish gelatine and mannitol.

In another preferred embodiment of the invention, the solid dosage form comprises starch and mannitol.

Detailed description of the invention

A non-compressed fast dispersing solid dosage form, which is designed to release the active ingredient almost instantaneously in the oral cavity on con-

tact with saliva is very suitable for the delivery of the allergens to the mucosa oromucosally. However, a priori the use of this particular dosage form for allergens is associated with severe problems. This type of solid dosage form is namely characterized by a low mechanical strength compared to compressed tablets due to the inherent nature of the non-compressed matrix, which is almost wafer-like and fragile. This may result in the release of residual particles containing the allergen during handling of the dosage form by the patient. This is especially detrimental when the active ingredient is an allergen, because the allergen can elicit an allergic reaction in a disposed person or induce an allergic reaction, that sensitisation or allergic response being dose dependent. Maximum allowable levels for environmental contamination in the form of e.g. allergen in dust have been proposed depending on the allergen in question as low as 2 micro gram major allergen per gram house dust. (Allergy. Principles and practice (1993, 4. ed.), Mosby-Year book, Vol. I page 520).

Such non-compressed fast dispersing solid dosage form, which are manufactured by removal of a liquid from a solidified system comprising matrix forming agents, active ingredient and other suitable are manufactured *in situ*. Due to the *in situ* manufacturing process i.e. removal of the solvent from a solidified system of the active ingredient and the matrix forming excipients in the final container, i.e. blister packs, it is not possible to coat the dosage form in order to seal it and thus preventing the release of residues from the dosage form. Moreover, coating the dosage form is not possible, because it would jeopardize the instantaneously release properties of the dosage form.

Thus, there exists a need for a fast dispersing solid dosage forms containing allergens, which quickly releases the allergen in the oral cavity, and where the dosage form at the same time is of such a mechanical robustness, so ideally no residues will be released from the dosage form to the environment during handling of the dosage form by the patient.

Further, there is the need for a fast dispersing solid dosage form containing allergens having sufficient chemical stability of the allergen to allow the manufacture, transportation, storage and especially patient handling.

The term "fast dispersing dosage form" refers to dosage forms which disintegrate in less than about 90 seconds, preferably in less than 60 seconds, preferably in less than 30 seconds, more preferably in less than 20, even more preferably in less than 10 seconds in the oral cavity, even more preferred in less than 5, most preferably in less than about 2 seconds of being placed in the oral cavity.

The term "stable" refers to dosage forms where the loss in allergen content of at least one major allergen according to the method described in example 1 is less than 50 % of the initial content.

The term "low friability" refers to the amount of residual material that is lost from the dosage form when it is subjected to an external force. The solid dosage form has a sufficient friability and robustness to be transported, stored and handled if the residual material lost contains less than 500 SQ-units per solid dosage form, more preferably less than 250 SQ-units per solid dosage form, most preferably less than 150 SQ-U per solid dosage form of the total allergenic content of the dose. For the purpose of the present invention, the friability may be measured by a method according to the present invention or may be measured by a modified method according to the European Pharmacopoïea.

The term "non-compressed" refers to a solid dosage form, which is manufactured by removal of a liquid from a solidified system comprising matrix forming agents, active ingredient and other suitable ingredients resulting in a an allergen comprised solid matrix.

The term "solid dosage form" refers to a unit dosage form, which is not a liquid, nor a powder, when it is administered in the oral cavity.

"Tensile strength σ " is calculated according to the following equation

$\sigma = 3Wa \times 9.8\ Nmm\text{-}2 / 2d^2b$

where w = Peak load to fracture (kgF)
a= distance between supports
d = thickness of the fast dispersing solid dosage form (mm)
b = diameter of the fast dispersing solid dosage form (mm)

"Peak load to fracture" means the peak force required to fracture a unit in a three point bend test using an appropriate instrument (e.g. CT5, Engineering Systems, 1 Loach Court, Radford Bridge Road, Nottingham NG8 1NA).

The term "oromucosal" refers to a dosage form that is placed under the tongue or anywhere else in the oral cavity that allows the active ingredient to come in contact with the mucosa of the oral cavity or the pharynx of the patient.

The term "allergen" refers to any naturally occurring protein or mixtures of proteins that have been reported to induce allergic, i.e. IgE mediated reactions upon their repeated exposure to an individual. Examples of naturally occurring allergens include pollen allergens (tree-, weed and herb- and grass pollen allergens), mite allergens (from e.g. house dust mites and storage mites), insect allergens (inhalant, saliva- and venom origin allergens), animal allergens from e.g. saliva, hair and dandruff from e.g. dog, cat, horse, rat, mouse, etc., fungis allergens and food allergens. The allergen may be used in the form of an allergen extract, a purified allergen, a modified allergen or a recombinant allergen or a recombinant mutant allergen, any allergen fragment above 30 amino acids or any combination thereof.

SQ-unit: The SQ-unit is determined in accordance with ALK-Abelló A/S´s "SQ biopotency"-standardisation method, where 100.000 SQ units equal the standard subcutaneous maintenance dose. Normally 1 mg extracts contains

between 100000 and 1000000 SQ-unit depending on the allergen source from which they originate and the manufacturing process used. The precise allergen amount can be determined by means of immunoassay i.e. total major allergen content and total allergen activity. In this field of expertise, there is no international accepted standardisation method. Hence, if extracts of other origins are used, they need to be standardised against an ALK-Abello A/S extract, which is a well-known procedure for the person skilled in the art. The subject matter is dealt with in "Allergenic extracts", H. Ipsen et al, chapter 20 in Allergy, principle and practise (Ed. S. Manning) 1993, Mosby-Year Book, St. Louis and Løwenstein H. (1980) Arb Paul Ehrlich Inst 75:122.

"Uniformity of content": shall mean the variation of the doses unit from the stated dose.

"Water content": shall mean the content of residual water in a solid dosage unit determined quantitatively using a Karl Fischer titration principle. This method is based on the principle that a given amount of I_2 leads to a transformation of an equivalent amount of water (European Pharmacopoïea 2.5.12)

As used herein "Water activity a_w" is the effective water in a sample. Water activity measurements are carried out using methods known to the person skilled in the art, for example chilled mirror dew point technology, relative humidity with sensors that change electrical resistance or capacitance or using a lithium chloride electrode:

a_w can be calculated according to the following equation

$$a_w = p/ps = ERH\ (\%)/100$$

where

p = partiale pressure of water vapor at the surface of the product

ps = saturation pressure, or the partial pressure of water vapor above pure water at the product temperature.

ERH = equilibrium relative humidity.

It has now surprisingly been found that it is indeed possible to manufacture a low friable non-compressed fast dispersing solid dosage form containing allergens, which is sufficiently robust and does not release hazardous amounts of residue upon handling by the patient. Moreover, it has surprisingly been found that these formulations are indeed stable at room temperature. This finding has significant importance for the handling procedures of the final product. Cold storage at the manufacturing plant, during transport or during storage at the pharmacy is often associated with high cost, since the cooling facilities have to be closely monitored and it is also very expensive to invest in reliable cooling facilities. Moreover, with respect to compliance of the patient, it is also preferable that the dosage form can be stored at room temperature. The European Pharmacopeïa monograph for Allergen Products states that the moisture levels should not exceed 5 % for freeze-dried products (i.e. allergen extracts in vials). It has surprisingly been found that even the dosage forms according to the invention having water content above the required maximum level of 5% are stable at room temperature. Without being bound to theory it may be explained by the fact that the excipients of the fast dispersing solid dosage form binds the remaining water in the dosage form and reduces the water activity of the allergen vaccine dosage formulation. Hence, by reducing the water activity of the formulation, it is possible to obtain a stable formulation with no degradation of the allergen, even though the water content is higher than maximum level of 5%, which is prescribed for allergen extracts in vials.

Stability of the solid dosage form in order to ensure a sufficient shelf life of the final product may me measured with reference to physical and chemical properties of the solid dosage form or its individual constituents.

Water activity is one important factor contributing to the shelf life of a product. It is well known that the water activity of a product affects growths of bacteria

as well as the stability, the potency and consistency of pharmaceuticals. Also protein stability is influenced significantly by water activity due to their relatively fragile nature. Most proteins must maintain conformation to remain active. Maintaining low water activity levels helps to prevent or entice conformational changes, which subsequently is important to ensure that a protein in the form of an allergen is stable. Also hydrolytic degradation of proteins whether caused by enzymes or not is affected by the water activity.

Water activity measurements are carried out by using methods known to the person skilled in the art for example chilled mirror dew point technology, relative humidity with sensors that change electrical resistance or capacitance or using a lithium chloride electrode

The water activity of a solid dosage form preferable does not exceed 25 % and preferably be between 0.1 % - 20%, more preferably between 0.5 – 15 %, more preferably 2 – 8%, most preferably between 4-7 %

The water content of a solid dosage form determined according to the method described in example 1 does preferably not exceed 25 % and preferably be between 0.1 % - 20%, more preferably between 0.5 – 15 %, more preferably 2 – 8%, most preferably between 4-7 %

Several laboratory tests are available for characterising an allergen. The most widely used techniques are sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), crossed immunoelectrophoresis (CIE) and Rocket Immuno Electrophoresis (RIE). The quantification of individual allergens may be performed by a variety of quantitative immunoelectrophoretic techniques (QIE), Radial Immune Diffusion (RIE) or by enzyme-linked immunosorbent assays (ELISA). The determination of total allergenic potency is most frequently performed by radio allergosorbent test (RAST), (LIA) or related techniques. ELISA-based techniques may also be used.

Guidance to the normally applied acceptable limit for test measuring biopotency are found e.g. in Note for Guidance on Allergen Product ; The European Agency for the Evaluation of Meicinal Product, CPMP_BWP_243_96.

Preferably for the purpose of this invention the stability of the active ingredient i.a. the allergen is assessed by means of potency measurements of the allergen like total allergen activity and major allergen content.

The "intial allergenic activity" or the "initial content of at least one major allergens" of a solid dosage form means the value as after the completion of the manufacturing of the solid dosage form.

Loss in total allergen activity according the method described in example 1 should preferably be less than 50 % of the total initial activity, more preferably less than 30 % of the total initial activity , even more preferably be less than 20 % of the total initial activity, most preferably less than 15 % of the total initial activity.

The classification of an allergen as a major allergen can be subject to several test. An allergens are commonly classified as major allergen if at least 25% of the patients shows strong IgE binding (score 3) and at least moderate binding (score 2) from 50% of the patients, the binding being determined by an CRIE (CRIE Strong binding i.e. visible IgE-binding on a x-ray film after one day, CRIE Moderat binding i.e. binding after 3 days, CRIE Weak binding i.e. binding after 10 dage). Strong IgE binding from at least 10% of the patients classifies the allergen as an Intermediate allergen and clearly specific binding from less than 10% of the patients gives a Minor allergen. Other methods may also be used in determining the IgE binding of for instance IgE-blots.

Loss in the allergen content of at least one major allergen according to the method described in example 1 is preferably less than 50 % of the total initial

content, more preferably less than 30 % of the total initial content, even more preferably less than 20 % of the total initial content, most preferably less than 15 % of the initial content.

In one embodiment of the solid allergen dosage form the loss in total allergen activity according the method described in example 1 is less than 50 % of the total initial activity.

In another embodiment of the solid allergen dosage the loss allergen content of at least one major allergen according to the method described in example 1 is less than 50 % of the initial content.

The term "stable" refers to dosage forms, which do not significantly change after manufacture with respect to physical and chemical properties e.g. potency of the allergen, mechanical robustness and organoleptical properties in order to ensure a sufficient shelf life of the final product.

Thus, the stability of the solid dosage form is preferably assessed by additional parameters, such as mechanical robustness like friability, tensile strength, peak load to fracture, stability of physical properties i.a. the dispersion time and stability of organoleptical properties like visual appearance of the dosage form.

These can be evaluated by e.g. measurements of Peak load to fracture or tensile strength of the solid dosage forms of the current invention. As it is apparent from the equation from which the tensile strength can be calculated , the tensile strength value obtained depend of a number of parameter, which are subject to variation e.g. thickness or diameter of the solid dosage form and will contribute to the variation of value. Therefore Peak load to fracture is believed to be a even more accurate parameter for evaluation of the robustness of the solid dosage units of the current invention

In order to ensure that the solid dosage form is sufficient robust during storage and when handled by the patient, the dosage form needs to have a certain resistance to external force, but at the same time ensure that the solid dosage form disintegrates quickly in the mouth.

In an embodiment the solid dosage form has a Peak load to Fracture not less than 0.05 Kgf and below 0.9 Kfg

In a further embodiment of the current invention the solid dosage form has a tensile strength less than 1.0 N/mm2.

Preferably fast dispersing dosage form disintegrates instantaneously or quickly in the mouth upon contact with the saliva in order to ensure maximum exposure of allergen to immune competent tissue of the mucosa before swallowing. In a preferred embodiment the solid dosage form disintegrates in less than about 90 seconds, preferably in less than 60 seconds, preferably in less than 30 seconds, more preferably in less than 20, more preferably in 15 seconds, even more preferably in less than 10 seconds in the oral cavity, even more preferably in less than 5, most preferably in less than about 2 sec.

In a preferred embodiment of the invention, the compositions of the invention are fast dispersing solid dosage forms comprising a solid network of the allergen and any water-soluble or water-dispersible matrix. The network is obtained by subliming solvent from a composition in the solid state, the composition comprising a solution of the allergen and the matrix. More preferably the network is obtained by lyophilization.

Pharmaceutically acceptable excipients forming part of the matrix in the fast dispersing solid dosage form according to invention are matrix forming agents and additionally other suitable excipients such as adjuvants, antacids, diluents, enhancers, mucoadhesive agents, flavouring agents, taste masking agents, preservatives, antioxidants, surfactants, viscosity enhan-

cers, colouring agents, pH modifiers, sweeteners etc. These excipients are all selected in accordance with conventional pharmaceutical practice in a manner understood by the persons skilled in the art of formulating allergen vaccines.

Matrix forming agents suitable for use according to the present invention include excipients derived from animal or vegetable proteins such as gelatines, dextrins and soy, wheat and psyllium seed proteins; gums such as acacia, guar, agar and xanthan; polysaccarides; starch and modified starch, alignates; carboxymethylcellulose; carrageenans; dextrans; pectins; synthetic polymers such as polyvinylpyrrolidone; and polypeptide/protein or polysaccharide complexes such as gelatine-acacia complexes.

Other matrix forming agents suitable for use according to the present invention include sugars such as mannitol, dextrose, lactose, galactose and trehalose; cyclic sugars such as cyclodextrin; inorganic salts such as sodium phosphate, sodium chloride and aluminium silicates; and amino acids having from 2 to 12 carbon atoms such as a glycine, L-alanine, L-aspartic acid, L-glutamic acid, L-hydroxyproline, L-isoleucine, L-leucine and L-phenylalanine.

The solid dosage form preferably comprises at least 50% W/W of at least one matrix forming agent of the dosing solution. The dosing solution being the non-solid formulation of the matrix forming agents, the allergen and other optional exhibients before the solidification step

In one embodiment of the invention the solid dosage form comprises 5-30% W/W, more preferably 5-20% W/W, even more preferred between 5-12 % W/W of at least one matrix forming agent of the dosing solution.

Need for dry matter content of the dosing solution will also depend on the dimensions of the tablet.

A fast dispersing solid dosage form comprising fish gelatine and mannitol as matrix-forming excipients has been found to be especially advantageous with respect to stability, visual appearance, low friability, tensile strength, peak load to fracture and mouth feel. In a preferred embodiment the fast dispersing solid dosage forms comprises a solid network of the allergen and matrix form agents in the form of fish gelatine and mannitol. In a preferred embodiment the content of fish gelatine is between 2-20 % W/W of the dosing solution and the content of mannitol is between 1-20 %W/W of the dosing solution. In another preferred embodiment the content of fish gelatine is between 2-10 % W/W of the dosing solution and the content of mannitol is between 1-10 %W/W of the dosing solution. In a further preferred embodiment the content of fish gelatine is between 3-6.5 % W/W of the dosing solution and the mannitol is between 3-5.5 % W/W of the dosing solution.

In yet a further embodiments the matrix comprises 4 % W/W of the dosing solution fish gelatine and 3 % mannitol W/W of the dosing solution

In another embodiment the matrix comprises 6.5 % W/W fish gelatine of the dosing solution and 5.5 % W/W mannitol of the dosing solution.

A fast dispersing solid dosage form comprising starch and mannitol as matrix-forming excipients has also been found to be especially advantageous with respect to stability, visual appearance, low friability, tensile strength, peak load to fracture and mouth feel. In a preferred embodiment the fast dispersing solid dosage forms comprises a solid network of the allergen and matrix form agents in the form of starch preferably pre-gelatinised from e.g. potato, wheat, maize, corn or rice and mannitol. In a preferred embodiment the content of starch is between 2-20 % W/W of the dosing solution and the content of mannitol is between 1-20 % W/W of the dosing solution. In another preferred embodiment the content of starch is between 2-10 % W/W of the dosing solution and the content of mannitol is between 1-10 % W/W of the dosing solution. In a further preferred embodiment the content of starch is

between 3-6.5 % W/W of the dosing solution and the mannitol is between 3-5.5 % W/W of the dosing solution

In another embodiment the matrix comprises 4.4. % W/W starch of the dosing solution and 4.4% W/W mannitol of the dosing solution.

Suitable colouring agents include red, black and yellow iron oxides and FD & C dyes such as FD & C blue No. 2 and FD & C red No. 40. Suitable flavouring agents include mint, raspberry, liquorice, orange, lemon, grapefruit, caramel, vanilla, cherry and grape flavours and combination of these. Suitable pH modifiers include citric acid, tartaric acid, phosphoric acid, hydrochloric acid and maleic acid. Suitable sweeteners include aspartame, acesulfame K and thaumatic. Suitable taste-masking agents include sodium bicarbonate, ion-exchange resins, cyclodextrin inclusion compounds, adsorbates or microencapsulated actives.

Adjuvants are normally used to enhance the absorption of the allergen as well as to enhance the immune-stimulating properties of the allergen.

In one embodiment of the invention at least one adjuvant is incorporated into the dosage form according to the invention. Examples of suitable adjuvants are aluminium salts, non-toxic bacterial fragments, cytokines, chlorea toxin (and detoxified fractions thereof), chitosan, homologous heat-labile of E.coli (and detoxified fractions thereof), saponins, bacterial products such as lipopoly-saccharides (LPS) and muramyl dipeptide (MDP), liposomes, CpG (immunostimulatory DNA sequences), lactide/glycolide homo ± copolymers in the form of microparticular polymers etc. The use of adjuvants in allergen vaccines are often reasoned by the fact the allergens in question are not able to penetrate the barrier to be passed. The adjuvants thus may serve as absorption enhancing agents or they may act as immunostimulants. The use of adjuvants may, however, be associated with serious draw backs such as unintended stimulation of various mechanisms of the immune response, systemic lupus erythematosus or affecting the barrier capabilities of the mucosal

membranes and thus allowing the passage of hazardous substances. Further from an industrial point of view addition of an adjuvant further constitute further manufacturing and material cost besides the large demand for documentation in respect to drug registration.

In another preferred embodiment of the invention the fast dispersing solid dosage form according to the invention does not comprise an adjuvant.

It has also surprisingly been found that it is not necessary to incorporate an adjuvant into the fast dispersing solid dosage form in order to enhance the immune-stimulating properties of the allergen in question i.e. that the solid dosage form is capable of raising a specific immune response cf. example 5.

A non-compressed fast dispersing solid dosage form may be mucoadhesive to some extent, however in a preferred embodiment of the invention, it may be necessary to further add mucoadhesive excipients to said dosage form in order to increase the contact time of the dosage form with the mucosa of the oral cavity. Suitable mucoadhesive excipients are polyacrylic polymers such as carbomer and carbomer derivatives; cellulose derivatives such as hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcelllulose and sodium carboxymethylcellulose; natural polymeres such as gelatine, sodium alginate, pectin and glycerol.

According to the invention an allergen vaccine is provided in a fast dispersing solid dosage form, which rapidly dissolves in the oral cavity on contact with saliva, hence bringing the allergen in close contact with the immunological relevant tissue of the mucosa and allowing the allergen to address these. In a preferred embodiment of the invention the allergen according to the present invention is any naturally occurring protein that has been reported to induce allergic, i.e. IgE mediated reactions upon their repeated exposure to an individual. Examples of naturally occurring allergens include pollen allergens (tree-, herb, weed-, and grass pollen allergens), insect allergens (inhalant, saliva and venom allergens, e.g. mite allergens, cockroach and midges aller-

gens, hymenopthera venom allergens), animal hair and dandruff allergens (from e.g. dog, cat, horse, rat, mouse,etc.), and food allergens. Important pollen allergens from trees, grasses and herbs are such originating from the taxonomic orders of Fagales, Oleales, Pinales and platanaceae including i.a. birch (Betula), alder (Alnus), hazel (Corylus), hornbeam (Carpinus) and olive (Olea), cedar (Cryptomeria and Juniperus), Plane tree (Platanus), the order of Poales including i.a. grasses of the genera Lolium, Phleum, Poa, Cynodon, Dactylis, Holcus, Phalaris, Secale, and Sorghum, the orders of Asterales and Urticales including i.a. herbs of the genera Ambrosia, Artemisia, and Parietaria . Other important inhalation allergens are those from house dust mites of the genus Dermatophagoides and Euroglyphus, storage mite e.g Lepidoglyphys, Glycyphagus and Tyrophagus, those from cockroaches, midges and fleas e.g. Blatella, Periplaneta, Chironomus and Ctenocepphalides, and those from mammals such as cat, dog and horse, venom allergens including such originating from stinging or biting insects such as those from the taxonomic order of Hymenoptera including bees (superfamily Apidae), wasps (superfamily Vespidea), and ants (superfamily Formicoidae). Important inhalation allergens from fungi are i.a. such originating from the genera Alternaria and Cladosporium.

In a more preferred embodiment of the invention the allergen is Bet v 1, Aln g 1, Cor a 1 and Car b 1, Que a 1, Cry j 1, Cry j 2 , Cup a 1, Cup s 1, Jun a 1, Jun a 2, jun a 3, Ole e 1 , Lig v 1, Pla l 1, Pla a 2, Amb a 1, Amb a 2, Amb t 5, Art v 1, Art v 2 Par j 1 , Par j 2, Par j 3, Sal k 1, Ave e 1, Cyn d 1, Cyn d 7, Dac g 1, Fes p 1, Hol l 1, Lol p 1 and 5, Pha a 1, Pas n 1, Phl p 1, Phl p 5, Phl p 6, Poa p 1, Poa p 5, Sec c 1, Sec c 5, Sor h 1, Der f 1 , Der f 2, Der p 1, Der p 2, , Der p 7, Der m 1, Eur m 2, Gly d 1, Lep d 2, Blo t 1, Tyr p 2, Bla g 1, Bla g 2, Per a 1, Fel d 1, Can f 1, Can f 2 , Bos d 2, Equ c 1, Equ c 2, Equ c 3 , Mus m 1, Rat n 1, Apis m 1, Api m 2 , Ves v 1, Ves v 2, Ves v 5, Dol m 1, Dil m 2, Dol m 5, Pol a 1, Pol a 2, Pol a 5, Sol i 1, Sol i 2, Sol i 3 and Sol i 4, Alt a 1, Cla h 1, Asp f 1, Bos d 4, Mal d 1, Gly m 1, Gly m 2, Gly m 3, Ara h 1, Ara h 2, Ara h 3, Ara h 4, Ara h 5 or shufflant hybrids from Molecular Breeding of any of these.

In the most preferred embodiment of the invention the allergen is grass pollen allergen or a dust mite allergen or a ragweed allergen or a cedar pollen or a cat allergen or birch allergen.

In yet another embodiment of the invention the fast dispersing solid dosage form comprises at least two different types of allergens either originating from the same allergic source or originating from different allergenic sources e.g. grass group 1 and grass group 5 allergens or mite group 1 and group 2 allergens from different mite and grass species respectively, weed antigens like short and giant ragweed allergens, different fungis allergens like alternaria and cladosporium, tree allergens like birch, hazel, hornbeam, oak and alder allergens, food allergens like peanut, soybean and milk allergens .

The allergen incorporated into the fast dispersing solid dosage form may be in the form of an extract, a purified allergen, a modified allergen, a recombinant allergen or a mutant of a recombinant allergen. An allergenic extract may naturally contain one or more isoforms of the same allergen, whereas a recombinant allergen typically only represents one isoform of an allergen. In a preferred embodiment the allergen is in the form of an extract. In another preferred embodiment the allergen is a recombinant allergen. In a further preferred embodiment the allergen is a naturally occurring low IgE-binding mutant or a recombinant low IgE-binding mutant.

Allergens may be present in equi-molar amounts or the ratio of the allergens present may vary preferably up to 1:20.

In a further embodiment of the invention the low IgE binding allergen is an allergen according to WO 99/47680 or WO02/40676 and in not yet published patent application "Allergen mutants" by ALK-Abelló A/S.

Preferably pH is adjusted prior to solidification of the allergen and matrix containing solution to avoid denaturation of the allergen, precipitation and assure a stable product. The optimum pH for different allergens in solution span al-

most the entire pH range as does their isoelectric point (pI). Mixtures of allergens like extracts equally have optimum for solubility and stability determined by factors like the concentration of the individual allergens in the extract. Therefore an individual determination of a feasible range of pH for a formulation according to this invention may be envisaged. The optimum pH for the allergen in question is determined by carrying out accelerated stability studies with formulations with different pH. The design of such studies is known to the person skilled in the art of formulating allergen vaccines.

Preferably matrix compositions containing an allergen extract should be adjusted to pH between 3.5- 10, more preferably 4-9, most preferably 6-9.

Furthermore it is well known in the art that ionic strength may be a parameter affecting the stability of a freeze-dried solid dosage form primarily through its effect of the freeze-drying processes. Also it is known to affect precipitation at high ionic strengths. Accordingly an optima must be established by measurements well known to the man skilled in the art. Preferably the ionic strength of an extract of 10 µg/ml is in between 1-1500 µS/cm, more preferably between 300 – 800 µS/cm, most preferably about 500 µS/cm, for a matrix and allergen containing system it is preferred that the ionic strength is between 1-2000 µS/cm, more preferably 500-1500 µS/cm.

Classical incremental dosage desensitisation, where the dose of allergen in the form of a fast dispersing solid dosage form is increased to a certain maximum relieves the symptoms of allergy. The preferred potency of a unit dose of the dosage form is from 150 – 1000000 SQ-u/dosage form, more preferred the potency is from 500 – 500000 SQ-u/dosage form and more preferably the potency is from 1000 – 250000 SQ-u/dosage form, even more preferred 1500-125000 SQ-u/dosage form most preferable 1500-75000 SQ-u/dosage form.

In another embodiment of the invention the vaccine is a repeated mono-dose, preferably within the range of 1500-75000 SQ-u/dosage form.

In further embodiment of the invention the allergen vaccine dissolved in saliva is not swallowed until 3 min after administration in order to allow sufficient contact time for e.g. absorption over the mucosal membrane in the mouth.

In yet a further preferred embodiment the allergen vaccine is not diluted in the oral cavity e.g. by intake of a fluid like water until after 5 min.

The fast dispersing solid dosage form according to the invention is manufactured and packed in disposable single dose blister packs as described in US 5,729,958 and US 5,343,762. Examples of suitable blister packs are All Aluminium Blister packs, blister packs made of polymers e.g. polypropylen, blister packs of PVC and blister packs formed from PVC/PVdC laminate and sealed with e.g. aluminium laminated to calendered kraft paper, Aclar® or Triplex®.

In an embodiment the fast dispersing dosage form is manufactured and packed in blister packs formed from PVC/PVdC laminate and sealed with aluminium laminated to calendered kraft paper. In another embodiment hereof the blister pack are enclosed in an aluminium sachet of suitable size, composed of aluminium laminated to calendered kraft paper.

In yet another embodiments the fast dispersing dosage form is packed in blister packs formed from aluminium and sealed with aluminium laminated to calendered kraft paper.

In a further embodiment the fast dispersing dosage form is packed in multilamilar blister packs formed from e.g. five layer aluminium laminate and sealed with aluminium laminated to calendered kraft paper.

In yet another embodiment the fast dispersing dosage form is packed in blister packs formed from aluminium laminate and sealed with aluminium laminated to calendered kraft paper in such a way that is difficult for children to open the blister pack e.g. child resistant packs

A solid dosage form of this type is normally characterized by a low mechanical strength compared to compressed tablets, because of the inherent nature of such a non-compressed dosage form. This may result in the release of residual particles containing the allergen on removal from the blister pocket and during handling of the dosage form by the patient. In most situations this is of no or mainly cosmetic importance, However, this is especially detrimental when the active ingredient is an allergen, because low amounts allergen can elicit an allergic reaction in a disposed person or sensitise. Normally exposure is in the range of 10 µg/year to major allergen protein accumulated for e.g. pollen allergens or dust mite allergens, which are adequate to give sensitisation or symptoms.

Upon handling the solid dosage forms, allergens may come in contact with target organs like the airways or the eye and elicit a response in an allergic person. One dosage form may contain as much allergen as a person is exposed to over one year or more depending upon the nature of the exposure. It is possible to induce eye symptoms in allergic patients using a conjunctival allergen challenge. Based on such challenge studies it can be estimated how much allergen extract is needed to induce conjunctival symptoms. In a population of patients with severe grass-pollen induced hayfever, the lowest dose of grass pollen extract causing conjunctival symptoms was proposed to be 3000 SQ-U/ml x 0,05 ml = 150 SQ-U (median value) (S. R. Durham, S. M. Walker, E. M. Varga, M. R. Jacobson, F. O'Brien, W. Noble, S. J. Till, Q. A. Hamid, and K. T. Nouri-Aria. Long-term clinical efficacy of grass-pollen immunotherapy. N.Engl.J.Med. 341 (7):468-475, 1999).

Thus, in one embodiment less than 500 SQ-U may be released from each solid dosage form during manual handling, more preferably less than 250 SQ-U, most preferably less than 150 SQ-U.

In order to ensure that allergen containing residues from the solid dosage form is not released to the environment upon opening the blister pack, it is important that the friability of the dosage form is as low as possible without jeopardising the allergen release from the dosage form following oral administration.

In a preferred embodiment of the present invention the residual content of dust in the blister pack after removing the dosage form do not exceed 2 % of total allergen content, more preferred 0,5 % of total allergen content of a solid dosage form and more preferably 0,2 % of total allergen content of a solid dosage form and most preferably 0,1 % of total allergen content of a solid dosage form.

Normally friability testing of compressed tablets is preformed as set out in the Pharmacopeia E.P. 2.9.7 and USP <1216>, wherein loss of weight is assessed as a parameter of an intact dosage form. Accordingly, the intactness of the current dosage form may be assessed by visual inspection and measurement of tablet weight upon having been subject to such a method. Alternatively, due to the low weight of dosage forms according to the invention the weighing can be replaced with an immune assay specific for the allergen in question.

The use of a modified friability test has been found to be a useful tool in assessing which compositions are most stable with respect to robustness and mechanical strength. In an embodiment the friability of said solid dosage form measured as the amount of allergen released is less than 500 SQ-U per solid dosage form, more preferably less than 250 SQ-U per solid dosage form,

most preferably less than 150 SQ-U per solid dosage form in any suitable friability test that exerts a sufficient external force on the compositions to be tested. In a more preferred embodiment the friability measured as the amount of allergen released is less than 500 SQ-U per solid dosage form, more preferably less than 250 SQ-U per solid dosage form, most preferably less than 150 SQ-U per solid dosage form in a friability test performed according to the Pharmacopoïea. In an even more preferred embodiment the friability measured as the amount of allergen released is less than 500 SQ-U per solid dosage form, more preferably less than 250 SQ-U per solid dosage form, most preferably less than 150 SQ-U per solid dosage form in an assay comprising the following steps;

a) placing individual units of solid dosage form contained in sealed blister pack unit in an equipment suitable for friability measurements
b) moving it for an appropriate time and at an appropriate velocity
c) removing the sealed solid dosage form unit
d) opening the sealed solid dosage form unit and emptying the unit content in a container/ placing the fast dispersing dosage form unit and any residues in a container
e) removing the solid dosage form unit from the container leaving any loose residuals in said container
f) performing an allergen specific assay on said residues determining the allergen content in said residues
g) optionally calculating the percentage allergen content in said residues of the total allergen content of the solid dosage form unit.

In a preferred embodiment of the friability method the units are rotated 100 turns at 25 ±1 rpm and the allergen content is determined by an ELISA assay.

Furthermore, the oral dosage form must have an appealing appearance. Hence, as a part of the quality control the fast dispersing solid dosage forms according to the invention are preferably subjected to visual inspection e.g colour, shape, irregularities and defects

In order to ensure optimum compliance of the patient, i.e. the patients perceive the dosage form as being pleasant when it is placed in the mouth and allowed to disintegrate, the dosage form may also be tested for mouth feel.

As allergens are very bio-potent for the allergic person i.e. even small amount may trigger a response, uniformity of content is an important parameter during treatment e.g. to ensure that a pattern experienced for a patient is reproducible when taken the same dose. Preferably the variation of content of allergen of units within a blister pack is within ±10%, preferably within ±7%, most preferable within ±5 % compared to the dose set.

A blister pack may contain any conceivable number of fast dispersing solid dosage forms. In preferred embodiment the blister pack contains 1-100 solid dosage forms and more preferably most preferably 5-35 solid dosage forms. The blister packs may further be packed in appropriated containers in accordance with any particular dose regime that is required to desensitise a patient.

The fast dispersing solid dosage form according to the invention can be prepared by a sublimation process according to the process disclosed in U.S. patent No. 4, 371,516. Accordingly, a solidified solution of the allergen and the matrix forming excipients is subjected to sublimation. The sublimation process is preferably carried out by freeze-drying the solution. The solution is contained in a depression of the blister pack during the freeze-drying step to produce a solid form in any desired shape. The blister pack can be cooled using liquid nitrogen or solid carbon dioxide. After the freezing step the frozen

solution in the blister pack is subjected to reduced pressure and, if desired, controlled application of heat to aid the sublimation of the solvent.

Clinical allergy manifestation and symptoms are several and may vary depending on the sensitized individual and the allergy inflicted. Common are symptoms like edema, itching, redness and running of the eyes and nose (rhinitisconjunctivitis) and symptoms from upper and lower airway like wheezing, coughing, shortness of breath, skin condition like eczema, urticaria and itching. Other symptoms like fatigue are also experienced. Symptomatic treatment aims at reducing or affecting severity of the symptoms or reducing the need for other drugs given in parallel. Symptomatic drug includes antihistamines like H_1 and H_2 receptor antagonists, intranasal and systemic corticosteroids, non-steroid anti-inflammatory drugs, nasal decongrdtans like adrenoceptor agonists. Treatment and relief of one or more allergic symptom or the reduction in the need for other medication is a further object of this invention.

Thus, a further object of the invention is to provide a method for treatment of allergy or alleviating symptoms of allergy comprising oromucosal administration of an effective amount of an allergen vaccine dosage form comprising (a) a matrix, and (b) at least one allergen in any of the above described embodiments.

Also a method for treatment of allergy or allergic symptoms comprising oromucosal administration of an effective amount of an allergen vaccine dosage form comprising (a) a matrix, and (b) at least one allergen further comprising an anti-allergic drug e.g. antihistamines in any of the above described embodiments.

The invention further include the use of an allergen for the manufacture of a stable, fast dispersing low friable, non-compressed allergen vaccine solid

dosage comprising (a) a matrix, and (b) at least one allergen form for use in the treatment of allergy or alleviating symptoms of allergy.

Further the invention provides a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form comprising (a) a matrix, and (b) at least one allergen for oromucosal treatment of allergy or allergic symptoms and the use of said dosage form for treatment of allergy or allergic symptoms.

In a further embodiment of the invention use of an allergen for the manufacture of a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form comprising (a) a matrix, and (b) at least one allergen for oromucosal treatment of allergy or allergic symptoms is comprised.

In yet another embodiment of the invention a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form comprising (a) a matrix, and (b) at least one allergen further comprising an antihistamine is used for oromucosal treatment of allergy or allergic symptoms.

Another object of the invention is to provide a method of producing a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form for oromucosal administration comprising

(a) a matrix

(b) at least one allergen

by

a) preparing an aqueous solution of the allergen and at least one matrix forming agent and optionally one or more suitable excipients

b) introducing the solution into depressions of a multilayer laminated blister sheet

c) subjecting the loaded sheet to freezing and freeze-drying using standard conditions of shelf temperature and chamber pressure.

Yet another object is to provide a method of obtaining a stable, fast dispersing, low friable, non-compressed solid allergen vaccine dosage form suitable for oromucosal administration comprising

1) producing a stable fast dispersing, non-compressed solid allergen vaccine dosage form
2) measuring the friability of said dosage form in an assay comprising the steps of

a) placing a solid dosage form contained in a sealed blister pack unit in an equipment suitable for friability measurements
b) moving it for an appropriate time and at an appropriate velocity
c) removing the sealed solid dosage form unit
d) opening the sealed solid dosage form unit and emptying the unit in a container/ placing the fast dispersing dosage form unit in a container
e) removing the solid dosage form unit from the container leaving any loose residues in said container
f) performing an immunochemical allergen specific assay on said residues determining the allergen content in said residues
g) calculating the percentage of allergen content in said residues in comparison to total allergen content of the solid dosage form unit
h) detecting whether the dosage form fulfills the requirements for low friability.

3) repeating 1) and 2) until the requirements for the dosage form is fulfilled.

Sublingual immunotherapy can be regarded as a a way of inducing tolerance inducing mucosal vaccination. The mucosa of the mouth is rich in dendritic cells with a strong potential for antigen presentation. The dendritic cells are believed to process the allergens and then migrate to the local lymph nodes

where they present allergen derived peptides to allergen specific T cells. During sublingual immunotherapy this dendritic cell - T cell interaction is believed to induce T cells with regulatory potential or to increase the ratio of allergen specific Th1 cells to allergen specific Th2 cells. A number of immunological parameters monitored during the allergy vaccination may be suitable markers for effects or efficacy of the treatment, alone or in combination respectively. These include systemic and mucosal antibody responses e.g. specific IgA, IgG and IgE antibodies; cytokine levels e.g. INFgamma, IL-2, IL-4, IL-5, IL-10, IL-12 and TNF alpha in blood or mucosal secretions; activation, chemotaxis, proliferation, signalling, cytokine production and other responses of regulatory T-cells, Th1 cells, TH2 cells, CD8 cells, other T cell subsets or B-cells or NK cells, and cell surface marker expression such as CD (cluster of differentiation) markers e.g. CD4, CD8, CD23, CD25, CD62L, CLA, beta7, CCR9, CD69, CD45RO, CCR3, CXCR5, effector cell function such as total histamine content of basophils; eosinophil, basophil, lymphocyte, monocyte numbers in blood, tissue and secretions; eosinophil, basophil, lymphocyte, monocyte mediator release, cytokine production, activation, chemotaxis, proliferation, signalling and other responses.

In a preferred embodiment a vaccine according to the present invention has a profile where one or more of the following immunological changes can be found; an increased allergen specific IgG response, an increased allergen specific IgA response, reduced allergen specific IgE response, few local side effects; reduced allergen specific effector responses of eosinophils, basophils, lymphocytes and/or monocytes; induction of T cells with regulatory potential, increased ratio of allergen specific Th1 cells to allergen specific Th2 cells, induction of other cells with regulatory potential, reduced allergen specific Th2 response.

Allergy is also a known disease in animals especially domestic and companion ship animal. It is known in the art that they develop allergies toward nu-

merous allergen sources including grass, house dust mites, and parasites. Hematopgagus, i.e. bloodsucking insect infestation is known to lead to a hypersensitive response called flea allergic dermatitis (FAD). In a preferred embodiment of the current invention allergens for animal vaccines include allergens originating or transferred from parasites like ectoparasites (e.g. fleas, ticks, mosqistos, flies), parasitic helminth venom (like hearth worm e.g. Dirotilaria or onchocerciasis e.g. Onchocerca) and house dust mite. More preferred are saliva allergens from fleas like Ctenocephalides e.g. C. canis and C. felis, hard ticks likes Ixodes, Arnblyomma, soft ticks like Ornithodoros and from midges like Culicoides.

Examples

Abbreviations;

API; Active Protein Ingredient

ELISA; Enzyme Linked Immuno Sorbent Assay

DDT: Dithiothreitol

HRP; Horse Radish Peroxidase

LIA; Magic Lite specific IgE assay

LITE-reagent: Luminescence labbelled anti-gE

PMP: Para Magnetic Particles

SDS-PAGE ; Sodium dodecyl sulphate poly-acryl amide gel electrophoresis

TMB; Tertametylbenzidine

Example 1. Allergen vaccine containing Phleum pratense grass pollen extract and fish gelatin.

Composition:

Table 1

Ingredients	Unit	Dosage form 1	Dosage form 2	Dosage form 3	Function	Reference to standards
Drug substance:						
Phleum pratense	SQ-U	2500	25000	125000	API	HSE
Other ingredients						
Purified water	mg	q.s to 250 mg	q.s to 250 mg	q.s to 250 mg	solvent	EP/USP
Gelatine (fish source)	mg	10	10	10	Matrix	EP/USP
Mannitol	mg	7.5	7.5	7.5	Matrix	EP/USP
Sodium hydroxide	mg	q.s	q.s	q.s	pH adjustment to 7.5	EP/USP

Grass extract

Grass pollen extract was prepared according to the method describes in Ipsen and Løwensten (1983) Jour. Allergy. Clin. Immunol. 72:2, page 150-159. In short grass pollen was extracted in ammonium hydrogen carbonate, for 20 hours at 5° C. Particulate matter was removed by centrifugation and the supernatant was dialysed against water (3 times), lyophilised and stored cold until reconstitution.

Solid dosage:

Manufacturing process:

1. The mannitol was added to an aliquot of the purified water (not less than 50% of the total batch requirement) and allowed to dissolve.

2. The gelatin was added to the mannitol solution and the solution was stirred on a magnetic stirrer until the gelatin had fully dissolved.

3. A second aliquot of the purified water (not more than 35% of the total batch requirement) was used to reconstitute the allergen extract in the vials. The reconstituted allergen extract was added to the mannitol-gelatin solution.

4. The pH of the bulk formulation was adjusted to pH 7.5 using freshly prepared sodium hydroxide solution (3% w/w).

5. The additional amount of purified water required to complete the formulation was calculated and transferred to the bulk mix.

6. The solution was dosed into pre-formed blister packs. The solutions were dosed under ambient temperature conditions.

7. After dosing, the filled blister packs were passed through a liquid nitrogen freeze tunnel. All frozen products were immediately placed in a frozen storage, prior to freeze-drying. The units were freeze-dried using standard conditions of shelf temperature and chamber pressure.

8. The freeze dried units were sealed with a lidding foil and finally packed in a sachet

Short descriptions of analytical methods:

Identity (ID), protein profile: The protein profile was determined by SDS-PAGE on a Novex Mini Cell Xcell II system (Invitrogen) according to manufacturers instructions. In short, samples are diluted with sample buffer added reducing agent (0.5 M DDT), and subjected to 70 ° C for 10 min and let to cool for 5 min. Sample, in-house reference and standard low-range size marker (by BIO-RAD) per well are applied on a NuPAGE 4-12 % Bis-Tris gradient gel. Electrophoresis is performed at 200 V for approximately 35 min. Subsequently the gel is stained with silver stained. The protein pattern has to be similar to that of the In house reference.

Visual inspection

All units were subjected to visual inspection e.g colour, shape, irregularities and defects to ensure acceptable appearance.

Disintegration: The test was performed as described in the current European Pharmacopoeia or the current USP.

Water content: The residual water was determined using a Karl Fischer titration principle. The method gives a quantitative determination of the water content in a sample based on the principle that a given amount of I_2 leads to transformation of an equivalent amount of H_2O.

Total allergenic activity: The test was performed using LIA (described in Eiken et all, Allergy 1992, 47:495-497), which is a competitive immunoassay 100 µl anti human IgE monoclonal antibody bound to paramagnetic particles (PMP) (ADVIA Centaur PMP, ALK-Abelló A/S, Denmark) are washed x 3 and added 100µl of a pool of patient sera with specific Phleum pratense IgE antibodies and are incubated on a shaker for 2 hours at 2-8 C °, whereby spe-

cific IgE binds to the PMP. The PMP are washed to remove IgG antibodies x 3 with gelatine buffer. 10 solid dosage form are dissolved in gelatin buffer and dilutions are prepared of 625 SQ-units or 1250 SQ-units per tablet. Samples or references of a known content of biotinylated Phleum pratense API are applied and incubated overnight on a shaking at 2-8° C. The samples and the biotinylated API will compete for the IgE binding sites, when the concentration of allergen in the sample rise, the amount of bound biotinylated API will drop. After incubation the samples are wash x 3 in gelatine buffer, and LITE- reagent i.a. streptavidin coupled acridinium ester chemiluminescent compound (ADVIA Centaur Lite Reagens, ALK-Abelló A/S is applied.. The samples are incubated for 2 hours on a shaker at 2-8 ° C, washed in gelatin buffer x 3 and read in a luminometer. The response is inversely related to the concentration of the allergen in the sample.

Major allergen content: The test was performed using ELISA technique. The ELISA method measures the concentration of Phleum pratense major allergen (Phl p 5) 5. Two monoclonal antibodies reacting with different epitopes on the phl p 5 molecule were coated to the microtiterplate. After washing and blocking the plate the sample/reference was applied and which then binds to the antibodies. After washing again biotinylated rabbit polyclonal antibodies against Phleum pratense antigens were applied to the well.

After wash is streptavidin coupled to HRP (horse radish peroxidase) applied to the wells. Streptavidin will bind to biotin on the polyclonal antibodies. After washing substrate (TMB) for the HRP enzyme was applied and the development of colour is proportional to the concentration of HRP in the well, which was equivalent to the concentration of phleum p 5 in the sample.

Friability:

The friability of the fast dispersing dosage forms was carried out using the following method.

Solid dosage form contained in a sealed blister pack were cut into individual dosage units and was placed in an equipment suitable for friability measurements as described in European Pharmacopoïea V. 2.9.7 the units were rotated 100 turns at 25 ± 1 rpm. The units were removed, opened and the solid dosage form was transferred to a suitable container. The solid dosage form was then removed from the container leaving any loose residues in said container. An immunochemical allergen specific assay (ELISA) was carried out to detect the amount of allergen content in the residues. It should be noted that when conventional dosage forms, such as tablets, are tested in a friability test according to the European Pharmacopoïea the tablets are not contained in a blister pack separated from each other, but allowed to rotate together.

Stability results:

Table 2

Product	**Dosage form containing *Phleum pratense* 2500 SQ-U**					
Storage condition:	**25°C/ 60% RH**					
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	Water content (%)	Visual inspection	Major aller-gen content (%)	Total allergenic activity (%)
Sampling (month)						
Start	0.000	8	5.5	Comply	96	101
1	n.m.	5	4.9	Comply	79	91
2	n.m.	6	5.4	Comply	96	102
3	0.000	5	5.2	Comply	94	82
6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.000	5	5.3	Comply	83	105
Storage condition:	**40 °C /75% RH**					
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	Water content (%)	Visual inspection	Major allergen content (%)	Total aller-genic activ-ity (%)
Sampling (month)						
1	n.m.	3	4.8	Comply	85	86
2	n.m.	8	5.2	Comply	85	100
3	0.005	6	5.4	Comply	94	83
6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.005	4	5.1	Comply	70	92

Table 3

Product	**Dosage form containing *Phleum pratense* 25000 SQ-U**						
Storage condition:	**25°C/ 60% RH**						
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water con-tent (%)	Visual inspection	Major allergen content (%)	Total aller-genic activity (%)
Sampling (month)							
Start	0.000	8	Comply	6.3	Comply	106	104
1	n.m.	n.m.	Comply	n.m.	n.m.	n.m.	n.m.
2	n.m.	n.m.	Comply	n.m.	n.m.	n.m.	n.m.
3	n.m.	n.m.	Comply	n.m.	n.m.	n.m.	n.m.
6	n.m.	7	Comply	n.m.	Comply	87	141
9	0.000	4	Comply	5.1	Comply	79	105
Storage condition:	**40 °C /75% RH**						
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water con-tent (%)	Visual inspection	Major allergen content (%)	Total aller-genic activity (%)
Sampling (months):							
1	n.m.	n.m.	Comply	n.m.	n.m.	n.m.	n.m.
2	n.m.	n.m.	Comply	n.m.	n.m.	n.m.	n.m.
3	0.005	n.m.	Comply	n.m.	n.m.	n.m.	n.m.
6	n.m.	6	Comply	n.m.	Comply	84	135
9	0.005	4	Comply	5.0	Comply	77	105

Table 4

Product	**Dosage form containing *Phleum pratense* 125000 SQ-U**						
Storage condition:	**25°C/ 60% RH**						
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water content (%)	Visual inspection	Major al-lergen con-tent (%)	Total aller-genic activ-ity (%)
Sampling (months):							
Start	0.000	5	Comply	4.7	Comply	100	100
1	n.m	10	Comply	3.9	Comply	84	93
2	n.m.	5	Comply	4.5	Comply	92	104
3	n.m.	8	Comply	4.8	Comply	80	88
6	n.m.	6	Comply	n.m.	Comply	n.m.	n.m.
9	0.003	5	Comply	4.5	Comply	77	106
Storage condition:	**40 °C /75% RH**						
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water content (%)	Visual inspection	Major al-lergen con-tent (%)	Total aller-genic activ-ity (%)
Sampling (months):							
1	n.m.	4	Comply	4.1	Comply	90	92
2	n.m.	6	Comply	4.6	Comply	91	109
3	0.001	7	Comply	4.5	Comply	83	89
6	n.m.	5	Comply	n.m.	Comply	n.m.	n.m.
9	n.m.	5*	Comply	4.6	Comply	78	118

*Mean value of only 3 dosage forms

Example 2. Allergen vaccine containing Phleum pratense grass pollen extract and starch.

Composition:

Table 5

Ingredients	Unit	Dosage form 1	Dosage form 2	Dosage form 3	Function	Reference to standards
Drug substance: *Phleum pratense*	SQ-U	2500	25000	125000	API	HSE
Other ingredients Purified water	mg	q.s to 250 mg	q.s to 250 mg	q.s to 250 mg	solvent	EP/USP
Pre-gelatinised starch	mg	8mg	9mg	11mg	Matrix	USP/NF
Mannitol	mg	8mg	9mg	11mg	Matrix	EP/USP
Sodium hydroxide	mg	q.s	q.s	q.s	pH adjustment to 7.5	EP/USP

Manufacturing process:

Same as example 1, pre-gelatinised starch was added instead of gelatine (fish source)

Short descriptions of analytical methods:

Same as example 1.

Stability results:

Table 6

Product	**Dosage form containing *Phleum pratense* 2500 SQ-U**				
Storage condition:	**25°C/ 60% RH**				
Tests:	Friability % loss of total content of extract (API)	Disinte-Gration (sec.)	Water content (%)	Visual in-spection	Total allergenic activity (%)
Sampling (months):					
Start	0.008	8	3.5	Comply	101
1	n.m.	5	3.0	Comply	80
2	n.m.	6	3.6	Comply	99
3	0.021	5	3.9	Comply	69
6	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.010	5	3.5	Residues	101
Storage condition:	**40 °C /75% RH**				
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	Water content (%)	Visual in-spection	Total allergenic activity (%)
Sampling (months):					
1	n.m.	3	2.9	Comply	74
2	n.m.	8	3.7	Comply	98
3	0.022	6	4.3	Comply	73
6	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.003	4	n.m.	Residues	75

Table 7

Product	**Dosage forms containing *Phleum pratense* 25000 SQ-U**					
Storage condition:	**25°C/ 60% RH**					
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water content (%)	Visual in-spection	Total aller-genic activity (%)
Sampling (months):						
Start	0.022	10	Comply	3.3	Comply	106
1	n.m.	n.m.	Comply	n.m.	n.m.	n.m.
2	n.m.	n.m.	Comply	n.m.	n.m.	n.m.
3	n.m.	n.m.	Comply	n.m.	n.m.	n.m.
6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.010	160	n.m.	3.5	Residues	99
Storage condition:	**40 °C /75% RH**					
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water content (%)	Visual in-spection	Total aller-genic activity (%)
Sampling (months):						
1	n.m.	n.m.	Comply	n.m.	n.m.	n.m.
2	n.m.	n.m.	Comply	n.m.	n.m.	n.m.
3	n.m.	n.m.	Comply	n.m.	n.m.	n.m.
6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.050	60	n.m.	3.4	Residues	96

Table 8

Product	**Dosage forms containing *Phleum pratense* 125000 SQ-U**					
Storage condition:	**25°C/ 60% RH**					
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water con-tent (%)	Visual in-spection	Total aller-genic activity (%)
Sampling (months):						
Start	0.041	11	Comply	2.6	Comply	121
1	n.m	18	Comply	2.4	Residues	102
2	n.m.	30	Comply	3.0	Residues	126
3	0.055	28	Comply	3.6	Residues	102
6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	0.0030	58	n.m.	2.9	Residues	99
Storage condition:	**40 °C /75% RH**					
Tests:	Friability % loss of total content of extract (API)	Disinte-gration (sec.)	ID	Water con-tent (%)	Visual in-spection	Total aller-genic activity (%)
Sampling (months):						
1	n.m.	58	Comply	2.5	Residues	96
2	n.m.	25	Comply	3.1	Residues	121
3	0.033	25	Comply	3.5	Residues	97
6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	n.m.	70	n.m.	2.7	Residues	110

Examples 3. Allergen vaccine containing grass extract and fish gelatin.

Table 9

Ingredients	Unit	Dosage form 1	Dosage form 2	Dosage form 3	Function	Reference To standards
Active substance:						
Phleum pratense	SQ-U	2500	25000	75000	Active substance	HSE
Other ingredients						
Purified water	mg	q.s. to 250 mg	q.s. to 250 mg	q.s. to 250 mg	Solvent	Ph. Eur. and USP
Gelatine (fish source)*	mg	16	16	16	Matrix	Ph. Eur. and USP/NF
Mannitol	mg	14	14	14	Matrix	Ph. Eur. and USP
Sodium hydroxide	mg	q.s.	q.s.	q.s.	pH adjustment 7.5	Ph. Eur. and USP

Manufacturing process:

Same as example 1.

Short descriptions of analytical methods:

Same as example 1, friability and stability was not measured.

Results of analysis:

Table 10

Test Methods	Major allergen content (%) ELISA	Total allergenic activity (%) LIA	Disintegration (sec.)	Water content (%)
Strengths				
2500 SQ-U/dosage form	97	102	1	5.5
25000 SQ-U/dosage form	100	89	1	5.3
75000 SQ-U/dosage form	94	95	1	5.2

Results

As apparent from examples 1, 2, and 3 it is possible to manufacture fast dispersing solid allergen vaccine dosage forms which disintegrate instantaneously. The loss of total content of extract was found to be acceptable, so even though the visual inspection resulted in the detection of residues in some of the blister packs (predominantly to a higher degree for starch containing matrixes), the amount of the residue i.e. allergen content loss is within the acceptable limit. Thus, it is possible to manufacture low friable non-compressed fast dispersing solid dosage forms containing allergens.

The stability data show that the formulations are stable at room temperature and at elevated temperature and humidity for nine months

All manufactured batches were subjected to visual inspection and found within the acceptable.

Examples 4: Allergen vaccine compositions

Solid allergen vaccine dosage forms were prepared containing varying ratios of matrix forming agents.

Table 11: Solid dosage form containing 75000 SQ phleum pratense grass pollen extract prepared in different packs

% gelatin	% mannitol	pack type	Load to Fracture (Kgf)	Disintegration times (sec.)
4.00	3.00	5 layer foil	0.158	< 2
4.00	3.00	PVC/PVdC	0.199	< 2
5.00	3.75	5 layer foil	0.296	< 2
5.00	3.75	PVC/PVdC	0.264	< 2
6.00	4.50	5 layer foil	0.342	< 2
6.00	4.50	PVC/PVdC	0.386	< 2
7.00	5.25	5 layer foil	0.491	< 2
7.00	5.25	PVC/PVdC	0.421	< 2

All dosage forms were prepared in blister pack having a unit diameter of 12 mm as described previously. All dosage forms disintegrated rapidly and were robust as assessed by visual appearance, tensile strength and peak load to facture.

Table 12: Solid dosage forms containing 75000 SQ-units of phleum pratense grass pollen extract in fish gelatin and mannitol.

% gelatin	% mannitol	Tensile strength (Nmm^{-2})	Peak load to Fracture (Kgf)	Disintegration times (sec.
5	4	0,239	0,168	<2
6.5	5	0,361	0,265	<2
6.5	5.5	0,425	0,277	<2
5	7	0,389	0,239	<2
8	4	0,531	0,308	<2
8	7	0,708	0,465	<2
7	7	0,543	0,355	<2
7	5	0,458	0,311	<2
6	4	0,263	0,169	<2
6	7	0,381	0,265	<2

All dosage forms were prepared in blister pack having a unit diameter of 12 mm as described previously.

All dosage forms disintegrated rapidly and were robust as assessed by visual appearance and Peak load to fracture.

Example 4 Uniformity

Dosage forms according to composition and manufacture as described in example 1 where tested for uniformity of allergen content. The allergen content was determined as uniformity of potency of grass pollen phleum p 5 by an ELISA assay as described in example 1 for the dosage form containing 25000 and 125000 SQ-units respectively. 10 individual units from a blister pack were compared as shown in table 13 and 14.

Table 13. Uniformity of content of 25000 SQ-unit dosage form.

ID (dosage form no.)	% of total allergen content
1	97,0
2	98,6
3	97,7
4	95,6
5	97,2
6	99,3
7	95,7
8	96,9
9	97,4
10	98,3
mean value	97,4

Table 14. Uniformity of content of 125000 SQ-unit dosage form

ID (dosage form no.)	% of total allergen content
1	101,4
2	102,4
3	102,3
4	101,9
5	104,4
6	100,5
7	101,1
8	104,0
9	113,4
10	97,1
mean value	102,8

All variations were within acceptable and good uniformity of allergen content was found.

Example 5. Admininistration of a phleum pratense grass pollen vaccine to dogs.

Dogs were equally distributed in respect to sex within each study group and were dosed following according to table 15

Table 15:

Group assigment				
Group	dose level (in SQ-units)	Number of dosage forms[a]	Number of dogs	Number of recovery dog
1	0	1	8	4
2	25000	1	8	
3	500000	4	8	4
[a] Group 1 received placebo dosage forms, group 2 received 25000 and group 3 received 125000 SQ-unit dosage forms prepared according to example 1				

The dogs were administered doses as indicated in Table 15 sublingually. The dosage forms was placed under the tongue and the snout was held closed to allow dissolution of the dosage form. The animals were dosed once per day for a period of 4 consecutive weeks. Blood samples were drawn for all dogs in every group after the completion of the treatment period. 4 dogs in the placebo and the high dose group respectively continued through a recovery period of 4 weeks where after further blood sample were drawn.

Method:

Phleum pratense (Phl p) specific IgG in either serum or plasma was determined as follows: ELISA plates (Costar) is coated with 10 µg/ml Phl p extract over night at 4 °C. Plates are washed 4 times with 1 min soak in between and

blocked against unspecific binding with 2% Casein buffer for one hour at room temperature. Individual serum or plasma samples are diluted in polypropylene plates, transferred to the ELISA plates and incubated for two hours at room temperature. After washing, HRP marked anti-dog IgG (ICN) is added to the ELISA plates and incubated for one hour at room temperature. After another wash, TMB is added to the ELISA plates, covered and incubated for 20 min at room temperature. The reaction is stopped with 0.5M sulphuric acid. The absorbance (OD) is measured in a spectrophotometer at 450 nm.

The OD values at 1:200 dilution are compared for the dogs in the three groups: placebo, 25000 SQ/dose and 500000 SQ/dose. Statistical difference between the three groups is calculated with the Mann-Whitney rank sum test which is a nonparametric test that compares two unpaired groups. Dogs receiving 500000 SQ units had higher mean value than both 25000 and placebo indicating a specific antibody response.

Results:

P values from the Mann-Whitney test are depicted in Table 16.

Table 16

Groups	P value
Placebo vs 25000 SQ/dose	0.059
Placebo vs 500000 SQ/dose	0.004

P-level ≤ 0.05 = significant with a 95% certainty.

There is a clear significant difference between the placebo and the 500000 SQ/dose group indicating that s.l. treatment with 500000 SQ/dose for 4 weeks gives a higher humoral specific IgG level. There is a borderline significant difference between the placebo and 25000 SQ/dose group also indicating that treatment with 25000 SQ/dose gives a humoral specific IgG level, although weaker than treatment with 500000 SQ/dose.

Examples 6. Administration of a phleum pratense grass pollen vaccine to allergics

Allergic patients, both female and male aged 18-65 years, with a diagnosis of grass pollen allergy were administered sublingual doses as one, two or three single doses and/or as multiple doses of a grass pollen extract containing solid dosage form, according example 1, in a randomised, double-blind, placebo-controlled designed trial.

Safety/tolerability was assessed with progressing single doses. Single doses of placebo, 2,500, 25,000, 75,000, 125,000 and 375.000 SQ-U were administered stepwise in a dose-escalating fashion using combinations of placebo and active tablets (12 mm in diameter and approximately 18 mg dry weight) to give the required dose. Forty-seven patients with allergy to grass pollen were treated. The dosage forms were placed under the tongue and held there for 1 minute before swallowing. Eating and drinking was prohibited for 5 minutes after application of the solid dosage form. The patients were observed for 2 hours for symptoms. All side effects were recorded, and after each dose the patients recorded tolerability on a Visual Analogous Scale. The dosage form containing the doses were found to be well tolerated up to and including 125.000 SQ-U as adverse events were predominantly mild in severity and limited to 'itching' phenomena in the mouth and throat. Adverse events were also reported in the placebo group. 'Itching mouth' was reported more frequently with increased dose i.a. a progression of adverse events correlated with progressing doses.

Further, safety/tolerability of repeated doses was tested for three selected doses, 2,500, 25,000 and 75,000 SQ-U, and placebo. Forty-seven patients with allergy to grass pollen,, distributed in four groups of comparable size, received a daily sublingual dosage for a period of 8 weeks. Combinations of three tablets containing placebo, 2,500 and/or 25.000 SQ-U (12 mm in di-

ameter and approximately 18 mg dry weight) were applied to obtain the required doses. Adverse events were recorded and symptoms were collected in patient diaries. The doses contained in the dosage form were found to be well tolerated in all three active treatment groups. Adverse events and symptoms were reported more frequently with increased dose.

Thus, the solid dosage forms tested are feasible for clinical use in both escalating dose and singular repeated dose therapy

Example 7. Allergen vaccine containing Phleum pratense grass pollen extract and fish gelatine.

Composition:

Table 17

Name of ingredients	Unit	Quantity per tablet	Quantity Per tablet	Quantity Per tablet	Function	Reference to standards
Active substance:						
Phleum pratense	SQ-U	2 500 low	25 000 med.	125 000 high	Active substance	HSE
Other ingredients						
Purified watera	mg	q.s to 250 mg	q.s to 250 mg	q.s to 250 mg	Solvent	Ph. Eur./USP
Gelatine (fish source)	mg	10.0	10.0	10.0	Matrix	Ph. Eur./USP, NF
Mannitol	mg	7.5	7.5	7.5	Matrix	Ph.Eur./USP

Sodium hydroxide	mg	q.s	q.s	q.s	pH modifier, Ph adjustred to 7.5	Ph. Eur/USP

Solid dosage forms manufactured according to example 1 were stored for 12 month at 25°/60%RH and evaluated by measurement of visual inspection, disintegration, water content, uniformity of mass, identity (protein profile), major allergen content and total allergenic activity. All test were performed as described in example one. Average of double analyses for pool of 10 tablets are shown unless otherwise stated.

Table 18.

Batch number 0000040914							
Storage conditions: 25°C/60% RH							
Product:	**2500 SQ-U/tablets**						
Test:	Visual inspection	Uniformity of mass	Disintegration (seconds)	Total allergenic activity. (LIA)	Major allergen content. (ELISA)	Friability	Water content (%)
0	Comply	comply	6	122%	97%	0.00%	6.1%
12	comply	Comply	1	105%	99%	N/A	4.8%

Product	25000 SQ-U/tablets							
Test	Visual inspection	ID (SDS-page)	Uniformity of mass	Disintegration (seconds)	Total allergenic activity. (LIA)	Major allergen content. (ELISA)	Friability	Water content (%)
0	Comply	Comply	Comply	5	108%	100%	0.00%	5.7%
12	N/A	Comply	N/A	N/A	N/A	N/A	N/A	N/A
Product	**125000 SQ-U/tablets**							
Test:	Visual inspection	ID (SDS-page)	Uniformity of mass	Disintegration (seconds)	Total allergenic activity. (LIA)	Major allergen content. (ELISA)	Friability	Water content (%)
0	comply	Comply	Comply	7	110%	99%	0.00%	5.4%
12	comply	Comply	Comply	1	119%	104%	N/A	4.2%

Visual inspection:

There was no change in the appearance of the tablets during the study.

Disintegration:

There was no remarkably change in the disintegration time during the study.

All test samples disintegrated immediately.

Uniformity of mass:

There was no remarkably change in the uniformity of mass during the study (assessment of 20 tablets).

Water content:

There was no remarkably change in the water content during the study.

Identity SDS-page:

There was no dramatic change in the protein profile during the study; the samples were similar to the reference at all test times.

Total allergenic activity:

No significant loss of total allergen activity was measured for the tablets (varies from 105%-119% of the theoretical content for the different strengths at 12 month, values which are within the deviation of the test performed).

Major allergen content:

No significant loss of allergen content as determined by major allergen content was measured for the tablets (varies from 99%-104% of the theoretical content for the different strengths at 12 month storage, values which are within the deviation of the test performed).

Claims

1. A stable, fast dispersing, low friable, non-compressed solid allergen vaccine dosage form suitable for oromucosal administration of allergen comprising

(a) a matrix

(b) at least one allergen

2. A dosage form according to claim 1, wherein the matrix comprises at least one matrix forming agent and optionally one or more additionally suitable excipients.

3. A dosage form according to claim 2, wherein the excipients may be selected from the group consisting of adjuvants, antacids, diluents, enhancers, mucoadhesive agents, flavouring agents, taste masking agents, preservatives, antioxidants, surfactants, viscosity enhancers, coloring agents, pH modifiers and sweetners.

4. A dosage form according to any one of claims 1 to 3, wherein the matrix comprises gelatine.

5. A dosage form according to claim 4, wherein the matrix comprises fish gelatine.

6. A dosage form according to any one of claims 1 to 3, wherein the matrix comprises starch.

7. A dosage form according to one of claims 1 to 3, wherein the matrix comprises mannitol.

8. A dosage form according to one of claims 1 to 3, wherein the matrix comprises fish gelatine and mannitol.

9. A dosage form according to claim 1-3, wherein the matrix comprises starch and mannitol.

10. A dosage form according to claim 8, wherein the matrix comprises 2-10 % W/W of the dosing solution fish gelatine and 1-10 % mannitol W/W of the dosing solution

11. A dosage form according to claim 8, wherein the matrix comprises 3-6.5 % W/W fish gelatine of the dosing solution and 3-5.5 % W/W mannitol of the dosing solution.

12. A dosage form according to claim 9, wherein the matrix comprises 3-6.5 % W/W starch of the dosing solution and 3-5.5 % W/W mannitol of the dosing solution.

13. A dosage form according to any one of claims 1 to 12 without an adjuvant.

14. A dosage form according to any one of claims 1 to 13, where the allergen is selected from tree pollen allergens, grass pollen allergens, mite allergens, insect allergens, venom allergens, animal hair and dandruff allergens and food allergens.

15. A dosage form according to claim 14, wherein the allergen is in the form of an extract, a purified allergen, a modified allergen or a recombinant allergen or a mutant of a recombinant allergen or any combination thereof.

16. A dosage form according to any of claims 14 -15, wherein the allergen is grass pollen.

17. A dosage form according to any of claims 14-15, wherein the allergen is dust mite.

18. A dosage form according to any of claims 1-15 comprising at least two different allergens.

19. A dosage form according to any of claims 1-18, wherein the potency is from 150 - 1000000 SQ-u/dosage form.

20. A dosage form according to any of claims 1-19, wherein the variation of content of allergen of units within a blister pack is ±10%, preferably ±7%, most preferable ±5 % compared to the dose set.

21. A dosage form according to any of claims 1-20 wherein the dosage form is for sublingual administration.

22. A dosage form according to any one of claims 1 to 21, wherein said solid dosage form disintegrates in less than about 90 seconds, preferably in less than 60 seconds, preferably in less than 30 seconds, more preferably in less than 20, even more preferably in less than 10 seconds in the oral cavity, even more preferably in less than 5 seconds, most preferably in less than about 2 seconds in the oral cavity.

23. A dosage form according to any one of claims 1 to 22, wherein said solid dosage form has a tensile strength less than 1.0 N/mm2.

24. A dosage form according to any one of claims 1 to 22, wherein said solid dosage form has a Peak load to Fracture not less than about 0.05 Kgf and below about 0.9 Kfg

25. A dosage form according to any one of claims 1 to 24, wherein said dosage form is sufficiently strong to be removed form a blister pack without releasing residues to the surroundings

26. A dosage form according to claim 25, wherein less than 500 SQ-U of allergen may be released from each solid dosage form during manual handling, more preferably less than 250 SQ-U of allergen may be released from each solid dosage, most preferably less than 150 SQ-U of allergen may be released from each solid dosage.

27. A dosage form according to any of claims 1-26 wherein the friability measured as the amount of allergen released is less than 500 SQ-U per solid dosage form, more preferably less than 250 SQ-U per solid dosage form, most preferably less than 150 SQ-U per solid dosage form in a suitable friability test

28. A dosage form according to claim 27, wherein the friability is measured in an assay comprising the following step;

a) placing single units of solid dosage form contained in sealed blisterpack unit in an equipment suitable for reproducible friability measurements
b) moving it for an appropriate time and at an appropriate velocity
c) removing the sealed solid dosage form unit
d) opening the sealed solid dosage form unit and emptying the unit in a container/ placing the fast dispersing dosage form unit in a container
e) removing the solid dosage form unit from the container leaving any loose residues in said container

f) performing an allergen specific assay on said residues and determining allergen content in said residues

g) optionally calculating the percentage of allergen content in said residues in comparison to total allergen content of the solid dosage form unit

29. A blister pack containing a number of a solid dosage forms according to any one of claims 1 to 27.

30. A method for treatment of allergy or alleviating symptoms of allergy comprising oromucosal administration of an effective amount of an allergen vaccine dosage form according to any of claims 1 to 28

31. Use of an allergen for the manufacture of a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form.

32. A stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form according to claim 1 for oromucosal treatment of allergy or allergic symptoms.

33. Use of an allergen for the manufacture of a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form according to claims 1 for oromucosal treatment of allergy or allergic symptoms.

34. A method of producing a stable, fast dispersing low friable, non-compressed allergen vaccine solid dosage form for oromucosal administration comprising

(a) a matrix

(b) at least one allergen

by

a) preparing an aqueous solution of the allergen and at least one matrix forming agent and optionally one or more suitable excipients
b) introducing the solution into depressions of a multilayer laminated blister sheet
c) subjecting the loaded sheet to freezing and freeze-drying using standard conditions of shelf temperature and chamber pressure.

35. A method of obtaining a stable, fast dispersing, low friable, non-compressed solid allergen vaccine dosage form suitable for oromucosal administration comprising

1) producing a stable fast dispersing, non-compressed solid allergen vaccine dosage form
2) measuring the friability of said dosage form in an assay comprising the steps of

a) placing a solid dosage form contained in a sealed blister pack unit in an equipment suitable for friability measurements
b) moving it for an appropriate time and at an appropriate velocity
c) removing the sealed solid dosage form unit
d) opening the sealed solid dosage form unit and emptying the unit in a container/ placing the fast dispersing dosage form unit in a container
e) removing the solid dosage form unit from the container leaving any loose residues in said container
f) performing an immunochemical allergen specific assay on said residues determining the allergen content in said residues
g) calculating the percentage of allergen content in said residues in comparison to total allergen content of the solid dosage form unit

h) detecting whether the dosage form fulfills the requirements for low friability.

3) repeating 1) and 2) until the requirements for the dosage form is fulfilled.